Immunity, Vol. 4, 415–419, May, 1996, Copyright ©1996 by Cell Press

CD40 and Its Ligand in Host Defense

Minireview

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At the onset, it appeared that immunology had finally identified a molecule with a singular function in the regulation of humoral immunity. Early studies in mice and mutations in humans (hyper immunoglobulin M syndrome; HIM) all pointed an accusatory finger at the ligand for CD40 (gp39; CD40L) as the cardinal element in the initiation of humoral immune responses to thymus-dependent (TD) antigens (reviewed by Durie et al., 1994b). However, just as interleukin-2 (IL-2) and IL-4 have lost their identity as unique T cell and B cell growth factors, respectively, so CD40L is losing its identity as an effector molecule that is exclusively involved in the regulation of humoral immunity. A recent flurry of manuscripts underscore the importance of this ligand–receptor pair in host defense outside of its central function in the regulation of humoral immunity.

Cell-Mediated Immunity

CD40 and Immunity to Microbes

Using mice whose CD40L or CD40 gene was disrupted by homologous recombination, three reports have recently established that CD40L is required for protective cell-mediated immunity to Leishmania major and to Leishmania amazonensis (Campbell et al., 1996; Kamanaka et al., 1996; Soong et al., 1996). In the CD40L-defective mice backbred onto a genetic background normally resistant to progressive infection with L. major, the acquisition of a susceptible phenotype was observed (Campbell et al., 1996). The evaluation of the T cell priming in these mice showed that the T cells from the wild-type infected mice produced 100- to 150-fold more interferon γ (IFNγ) then T cells from CD40L$^{-/-}$ mice. Furthermore, splenocytes from CD40L$^{-/-}$ mice produced far lower levels of IL-12 upon antigen stimulation when compared with splenocytes from wild-type mice. The inability of CD40L$^{-/-}$ splenocytes to produce IL-12 resulted from the inability of CD40L$^{-/-}$ T cells to induce IL-12 and was not due to an intrinsic defect in the macrophage compartment. Finally, a causative relationship between CD40L-induced IL-12 production and disease progression was shown by the observation that IL-12 administration "cured" CD40L$^{-/-}$ mice of L. major infection.

Unlike L. major, where selected strains of mice are resistant to infection, most inbred strains of mice are susceptible to L. amazonensis infection. When the CD40L gene was disrupted, the susceptibility of mice to L. amazonensis was greatly increased (Soong et al., 1996). When the tissue parasite burden in wild-type and CD40L$^{-/-}$ mice was compared, the CD40L$^{-/-}$ mouse burden was 50- to 60-fold higher and this difference increased at later times during the infection. Immunohistochemical analysis of infected areas illustrated that in the CD40L$^{-/-}$ there was a reduced inflammatory response, highlighted by a reduction of organized granuloma formation, that increased appearance of poorly differentiated macrophages together with reduced ulceration and necrosis. As found in the study cited above, no gross changes in T or B cell populations could account for the altered susceptibility to infection by the parasite. Reduced IgG and IgE antibody titers to parasite antigens also were noted in the CD40L$^{-/-}$ mice. Assessment of T cell function in the CD40L$^{-/-}$ mice revealed a lowered T cell proliferative response to parasite antigens and a virtual absence of IFNγ and lymphotoxin–tumor necrosis factor production. This was similar to the defect in inflammatory cytokines observed in CD40L$^{-/-}$ mice infected with L. major. Interestingly, attempts to protect mice from infection with vaccination to L. amazonensis proved futile in the CD40L$^{-/-}$ mice, yet effective in wild-type mice. Central to the diminished resistance to L. amazonensis in the CD40L$^{-/-}$ is a reduced capacity of macrophages to exert antiparasitic responses such as nitric oxide (NO) production. However, unlike the studies cited above, studies with L. amazonensis showed that the production of IL-12 was comparable to wild-type mice. This lead the authors to conclude that the major contributing defect to increased susceptibility to L. amazonensis infection was diminished macrophage activation and NO production.

Early studies describing the defects in CD40L$^{-/-}$ and CD40$^{-/-}$ mice revealed that disruption of either the receptor or the ligand or, in fact, treatment of mice with a neutralizing anti-CD40L antibody, resulted in identical phenotypic changes in the humoral immune response. Similarly, it has been reported (Kamanaka et al., 1996) that CD40-deficient mice, like the CD40L-deficient mice, have an increased susceptibility to L. major, and cannot resolve the infection. As observed in the CD40L$^{-/-}$ mice infected with L. major, the CD40$^{-/-}$ mice produced lowered amounts of IFNγ and IL-12. In the CD40$^{-/-}$ mice, higher levels of IL-4 were observed. All three studies strongly suggest that in the absence of CD40 signaling, T cell responses are polarized towards a T helper (Th)2-type response.

CD40 and Macrophage Function

The studies cited above underscore the important role that CD40 and its ligand play in host defense, over and above the role that this ligand–receptor pair play in humoral immunity. Along with the increased susceptibility to Leishmania, there is also an increased susceptibility to Pneumocystis carrini. This increased susceptibility has been observed in three contexts. First, treatment of mice with anti-CD40L diminished their ability to clear Pneumocystis (Wiley and Harmsen, 1995). Second, HIM patients have a exceedingly high incidence of infection with this pathogen (Notarangelo et al., 1992). Finally, my colleagues and I have observed increased susceptibility when our entire colony of CD40L$^{-/-}$ mice succumbed to Pneumocystis infection.

The diminished function of macrophages in the absence of CD40 signaling may result due to a reduced inflammatory response from T cells. It is now recognized that CD40L induces polarity in the T cell compartment

through its ability to trigger macrophages or dendritic cells (DCs) to produce IL-12 in the presence of IFNγ (Shu et al., 1995). That is, when CD40L is adequately expressed, an effective loop for the induction of potent Th1-type inflammatory responses will be evoked. Further evidence in support of this scenario is provided by the results of anti-CD40L administration in a model of inflammatory bowel disease (IBD) (Stuber et al., 1996). Rectal instillation of an inflammatory hapten incites IBD, whose hallmarks are elevated Th1 cytokines, IL-12 production, and inflammatory pathology. Treatment with anti-CD40L antibody prevents the inflammatory response and encourages the priming of Th2-type cells. Consistent with an anti-inflammatory role for anti-CD40L, anti-CD40L prevents and appears to arrest the development and progression of experimental allergic encephalomyelitis (EAE), a T cell–mediated inflammatory response in the central nervous system (CNS) (Gerritse et al., 1996). In this context, histological evidence suggested that macrophages or microglia were the prominent CD40-bearing cells in the CNS of EAE mice or multiple sclerosis patients.

As stated above, the influence of CD40L on macrophage activities is likely broad. In addition to its apparent positive impact on the production of inflammatory cytokines, CD40 also plays a direct role in microbicidal activity of macrophages. In one study cited above (Soong et al., 1996), it was concluded that the lack of inducible NO activity was the leading cause of increased susceptibility to infection. Supportive evidence for an important role of CD40 in the regulation of NO comes from studies that directly implicate CD40L in triggering NO in macrophages in vitro (Tian et al., 1995; Stout et al., 1996). CD40L regulation of inducible NO production has also been observed in the context of allogeneic organ transplant, whereby anti-CD40L allowed the long-term survival of cardiac transplants and this long-term survival was correlated with the block by anti-CD40L of inducible NO synthase (Larsen et al., 1996). Beyond its influence on microbicidal activity, CD40L also induces macrophages to produce many key proinflammatory cytokines like tumor necrosis factor α, IL-1β, IL-6, and IL-8 (Kiener et al., 1995). Therefore, CD40L appears to be a multipurpose macrophage activator involved in the up-regulation of microbicidal activity, antigen presentation, and costimulation, as well as the induction of proinflammatory and inflammatory cytokines.

CD40 and APC Function

The role that CD40 plays in the regulation of antigen-presenting cell (APC) activity is best exemplified by studies using B cells as APCs. Resting B cells express meager APC activity. A number of studies have shown that triggering via CD40 is an effective means of increasing the APC activity of B cells, most likely through the up-regulation of B7-1 and B7-2 (Roy et al., 1994; Ranheim and Kipps, 1995). The role of CD40–CD40L in regulating the APC capacity of B cells has recently been documented by showing that the combined administration of anti-CD40L and allogeneic B cells can diminish allospecific cytotoxic T lymphocyte and mixed lymphocyte responses (Buhlmann et al., 1995). This observation was applied to a system of "donor-specific transfusion" tolerance, whereby pretreatment of mice with allogeneic B cells and anti-CD40L was used to induce transplant tolerance. Pretreatment of mice in this way rendered mice allotolerant and allowed the long-term engraftment of allogeneic pancreatic islet cells (Parker et al., 1995). The question emerges as to whether a CD40 signal is critical for other APCs, i.e., DCs, to provide adequate costimulatory activity. Certainly, evidence does exist that at least some DCs express abundant levels of B7-2 in vivo and that B7-2 is the primary ligand in DC-induced T cell costimulation (Inaba et al., 1994; Larsen et al., 1994). Nonetheless, numerous DC populations, like the Langerhan cells of the skin, have low levels of B7-2 until up-regulated expression is driven by cytokines or CD40 triggering (Peguet et al., 1995). Functional studies strongly implicate CD40 as an important mediator of APC function. First, in studies of graft-versus-host disease (GVHD), it has been shown that brief treatment with anti-CD40L prevents the generation of anti-host cytotoxic T cells and prevents the onset of disease (Durie et al., 1994a). All of the evidence to date in the GVHD system suggests that the alloreactive T cells are being rendered tolerant in the absence of CD40 signaling. In addition to this study, other studies have shown that $CD40L^{-/-}$ mice are defective in T cell priming to protein antigens in complete Freund's adjuvant. This study directly illustrated that the in vivo expansion of antigen-specific T cells was impaired in the absence of ligand (Grewal et al., 1995). Taken together, these data strongly suggest that CD40L may play an indirect role in T cell priming, via the induction of costimulatory activity. In our own experience, however, this appears not to be a hard and fast rule, since we have shown that one can prime helper T cells to sheep red blood cells in the absence of CD40L (Foy et al., 1993). Therefore, unlike the essential function of CD40L in TD humoral immunity, the capacity of CD40L in the regulation of APC function may be supplanted by other immune activities. For example, GM-CSF and IL-4 are potent inducers of B7-2 on dendritic cells and B cells, respectively (Hathcock et al., 1994), and may circumvent the need for CD40 triggering. This may be the reason why no apparent defects in delayed-type hypersensitivity have been commonly observed in HIM patients.

CD40 and the Regulation of Adhesion

Anti-CD40L has been shown to block the development of a number of T cell–mediated autoimmune diseases, including collagen-induced arthritis (Durie et al., 1993), autoimmune oophoritis (Griggs et al., 1996), and EAE (Gerritse et al., 1996). In each of these examples, a reduction in T cell infiltration into the target organ/tissue was noted. In at least some of these examples, the lack of infiltration can not be explained by a lack in T cell priming (Griggs et al., 1996). For example, it has been shown that in the presence of anti-CD40L, T cell priming to a autoantigenic peptide induces increases in T cell precursor frequency similar to that observed in its absence; yet no T cell infiltration into the target organ was observed (Griggs et al., 1996). Therefore, how may anti-CD40L interfere with the trafficking of primed T cells into target organs? Three papers have shown that CD40 can regulate the expression of adhesion molecules on endothelial cells (Hollenbaugh et al., 1995; Karmann et al., 1995; Yellin et al., 1995). Endothelial cells were shown

to express heightened levels of CD40 in inflammatory skin disease; it also was shown that triggering of CD40 induced increased leukocyte adhesion as well as the up-regulation of VCAM, ICAM, and E-selectin. Therefore, CD40L–CD40 interactions may play an important role in the extravasation of activated T cells into the target organ and their accumulation, as well.

CD40L and Its Impact on Inflammation

How can blockade of a single effector molecule exert such profound effects on cell-mediated immunity? It is clear that the lack of CD40L (or the administration of anti-CD40L) blocks multiple immune mechanisms that are responsible for the development of inflammation. First, lack of CD40L function either alters or diminishes the priming and expansion of T cells in response to antigen, and polarizes the course of T cell differentiation toward a Th2 response. This effect is due to the role of CD40L in the regulation of costimulatory molecules on APC, or in the regulation of IL-12 production, or both. Second, even though some effector T cells may be generated in the absence of CD40L, their ability to traffic to the appropriate target organ may be diminished. One would anticipate that the lack of CD40L will interfere with the localization of the effector cells in the target organ by prohibiting the up-regulation of adhesion molecules required for effector T cell diapedesis and accumulation. Third, even though some effector T cells may be identified within the target organ, the lack of CD40L would seriously compromise their ability to trigger macrophage functions optimally, like the production of pro-inflammatory cytokines and inflammatory cytokines, the up-regulation of costimulatory molecules, and other mediators of inflammatory pathology. In sum, the role of CD40L at all of these levels of cell-mediated immunity help to explain why one can so effectively interrupt the development of autoimmune disease and graft rejection by the administration of anti-CD40L and why mice lacking CD40–CD40L cannot mount effective cell-mediated immune responses to parasites.

New Issues Concerning the Role of CD40L in the Genesis and Maintenance of Germinal Centers and Somatic Mutation

CD40L and Its Role in Germinal Center Formation

The early studies in CD40L$^{-/-}$ and CD40$^{-/-}$ mice, and in mice treated with anti-CD40L, documented that primary and secondary humoral immune responses were severely impaired by the absence of CD40 signaling (Foy et al., 1993; Kawabe et al., 1994; Renshaw et al., 1994; Xu et al., 1994). In addition, germinal center (GC) formation in response to TD antigens was completely absent. The interpretations of these studies suggested that signals via CD40 were responsible for GC formation. Alternative insights into the formation of GCs have emerged from studies in CD40$^{-/-}$ mice. CD40$^{-/-}$ mice that were administered CD40Ig (so as to engage CD40L) form a limited number of small GCs. These data are interpreted to suggest that engagement of CD40L by CD40Ig transduces a costimulatory signal to T cells to elaborate an activity that contributes to GC formation (van Essen et al., 1995). This is in agreement with earlier in vitro data that also suggest that CD40L, when engaged by a multimeric receptor, can transduce a costimulatory signal to the T cell (Cayabyab et al., 1994). We have shown that one can effectively in vivo prime CD40$^{-/-}$ T cells with allogeneic B cells to levels observed with wild-type T cells, if the allogeneic B cells are first activated with CD40L in vitro. This argues against a mandatory role for CD40L engagement in the process of T cell priming, and supports the hypothesis that the function of CD40L in T cell priming is in the activation of the APC. The issue of whether the engagement of CD40L by its receptor directly costimulates T cell activation demands further study before there is a decisive answer.

CD40L and Its Role in GC Maintenance

A most intriguing finding concerning the role of CD40L is that the administration of anti-CD40L causes the abrupt disappearance of preëxisting GCs (Han et al., 1995). It has been well established that GC B cells acutely apoptose upon isolation and that CD40 ligation can "rescue" them from death (Liu et al., 1992). Therefore, the obvious reason why anti-CD40L treatment causes the loss of GCs is due to increased apoptosis. However, reported findings suggest that apoptosis is not the cause for the rapid disappearance of GCs. This leaves at least two possibilities. First, that anti-CD40L shuts off the influx of B cells into GCs; however, influx into the GC appears to end long before the onset of anti-CD40L administration. Alternatively, it is possible that loss of CD40L function in the GC causes B cells to emigrate to the bone marrow and terminally differentiate into plasma cells. This hypothesis would be consistent with a recent in vitro study that shows that the withdrawal of CD40L results in memory B cells terminally differentiating to plasma cells (Arpin et al., 1995).

If the function of CD40L is so critical for GC maintenance, which cells in the GC are expressing CD40L? Our own collaborative studies in immune mice did not detect CD40L in the GC of immune mice (Van den Eertwegh et al., 1993); however, other studies in human tonsil have indicated its presence (Lederman et al., 1992; Casamayor et al., 1995). Recently (Casamayor et al., 1995), it has been shown that in human tonsil, a subset of T cells contained preformed CD40L that could be mobilized rapidly upon activation. The T cells containing preformed CD40L were found predominately in the outer zone of the GC and could be important cells for the maintenance of GC integrity. Regulation of CD40L expression by these T cells may well regulate the tempo of the GC reaction.

CD40L and Somatic Mutation

With its prominent role in the regulation of B cell growth and differentiation, CD40L was a tempting candidate for triggering the somatic hypermutation of IgV genes. Since triggering of B cells through CD40 induces explosive B cell growth and differentiation (Banchereau et al., 1994), it was of interest to determine whether CD40 signaling and lymphokines also triggered B immunoglobulin hypermutation. Single naive B cells were cultured in vitro with anti-CD40 and IL-4 and somatic mutations in the heavy chain V region were assessed. While single B cells were shown to isotype switch to downstream isotypes, an increase in the somatic mutation in IgH V genes could not be documented (Galibert et al., 1995). An alternative system that appears to sustain

somatic mutation in vitro was recently reported (Kallberg et al., 1996). B cells that were triggered by immunization in vivo, were isolated after at least 10 days, then cocultured with an alloreactive Th clone. Under these conditions, there was an increase in the number of Vκ mutations. Interestingly, swapping the Th2 clone with CD40L was largely ineffective at inducing Vκ mutations. Collectively, these observations suggest that CD40L is neither sufficient to initiate nor sustain somatic mutations. The success of others (Decker et al., 1995) in inducing somatic mutation in splenic fragments may suggest that lymphoid architecture is a requirement, together with antigen-specific T cell help, for B cells to initiate somatic mutation.

Conclusion

Over the past year, we have seen the function of CD40L greatly expand from a molecule that was exclusively involved in the regulation of TD humoral immune responses, to a molecule that signficantly contributes to the inflammatory process. Whether the function of CD40L in the inflammatory process is essential, like its function in TD humoral immunity, demands critical attention. It is clear that at least some inflammatory responses are intact in CD40L-defective mice and in HIM patients. Therefore, the question emerges as to in which subset of inflammatory responses does CD40L play a role? Numerous studies have implied that CD40L plays an indirect role in the priming of T cells; yet it is unclear at this time whether T cells primed in the absence of CD40L are anergic, ignorant, or physically deleted. The in vitro studies on the potential role of this ligand in the regulation of endothelial cell function are provocative; yet critical studies in vivo are needed to evaluate whether CD40–CD40L interactions are involved in lymphocyte trafficking. With the expanding role of CD40 and its ligand, clear answers to its function in regulating the immune response may only be resolved by interfering with CD40 signaling in a tissue- or cell-specific fashion.

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